To: Peters, Emily (MPCA)[Emily.Peters@state.mn.us]; Swain, Ed (MPCA)[edward.swain@state.mn.us]; Monson, Phil (MPCA)[phil.monson@state.mn.us]
From: Erickson, Russell
Sent: Sat 2/15/2014 5:29:06 PM
Subject: RE: EC20s for pooled hydroponic data

This analysis was done very well. However, in examining the fit of the relationships to the data, I think my suggestion to do a pooled analysis did not work out well, which I have discussed with Ed over the phone. My suggestion to do a pooled analysis was based on an expectation (or hope) that the data sets could be superimposed and would follow the same relationship once normalized to scaling parameters. However, the need to have a lower asymptote different from zero, and various differences among the data sets at the high concentrations make the results unsatisfactory in this regard. The pooling constraints caused some data fits to be poorer than desirable, and the constraints of the basic model shape might also be causing some undesirable fits. The issue of ECp definition is also a problem – the "EC50" based on being midway between the upper and lower asymptotes is close to complete cessation of growth in one set.

So I feel that something needs to be done other than this pooled analysis. A first step would be to simply fit the same model (4-parameter logistic) to the individual data sets. This would at least provide the best individual EC50s for each set, showing how this relatively robust statistic varies among the sets. The EC50s should be based on A/2 – halfway between the control and zero growth because zero growth provides a more consistent and relevant reference point for growth effects. The regression analyses would provide equations from which the EC50 can be computed, but not its confidence limits. To get confidence limits, the logistic equation would have to be modified so that the desired EC50 is an actual model parameter. I can provide an equation that does that. I realize that each dataset is a rather sparse to support this kind of analysis and estimates will thus be uncertain, but I think these regressions are possible and will provide better information than the pooled analysis.

Another alternative would be to base conclusions about ECs on ANOVA rather than regression analysis, and thus avoid various problems with the formulation and meaning of the regression analyses (….. or at least supplement the regression analyses with information from an ANOVA). The individual ANOVAs for the datasets already establish the obvious – that exposures with initial sulfide concentrations of 20uM or more (even less based on average concentrations) cause large effects, but these ANOVAs don't assign significant effects to the ca 10uM treatment in each test. However, averaged across the three tests, the weight increase in the ca10uM treatment is about 30% lower than control treatments and about 20% lower than the 3-5uM treatments in the tests. The low sample size and the degree of data variability just don't make this level of effect detectible.

However, the statistical significance for these effects at ca 10uM might be demonstrated by conducting a combined ANOVA or ANCOVA across all tests (tripling the sample size). This should be done only on the control and the lowest two treatments, since the higher treatments are irrelevant to the issue of whether 10uM is an effect concentration. Because there is some variation in the sulfide levels between tests, the best structure for this analysis is unclear. But as a first cut, I treated the control, the 3-5 uM, and the ca 10uM treatments in each test as the same categorical variables and ran a simple 3x3 factorial ANOVA. I ran this with various growth measures and in all cases a significant effect of sulfide treatment was reported and a Dunnett's multiple comparison test reported the 10uM treatment to be significantly less than the control (significance levels were always in the 0.01-0.03 range).

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From: Peters, Emily (MPCA) [mailto:Emily.Peters@state.mn.us]
Sent: Friday, February 14, 2014 11:13 AM
To: Swain, Ed (MPCA); Monson, Phil (MPCA)
Cc: Erickson, Russell
Subject: EC20s for pooled hydroponic data

Phil and Ed,

Attached are the model summaries and plots we just discussed for the hydroponic data. I managed to fit the information for the 4 separate models on 4 figures. Let me know if you need anything clarified. Russ was immensely helpful in conducting these analyses (and I expect will continue to be). I was greatly appreciated his advice.

-Emily

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